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(54) Title: METHOD FOR CONSTRUCTING LIBRARIES OF PHENOTYPIC PROFILES

(57) Abstract

Methods are provided for use in constructing libraries of phenotypic profiles in a nematode worm such as *C. elegans*. The methods require measurement of identifiable characteristics of the worm and systematic scoring of these characteristics. Also provided are methods of identifying compounds with potential pharmacological activity, for determining the mode of action of a given compound and for assigning genes to particular biochemical pathways.

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METHOD FOR CONSTRUCTING LIBRARIES OF PHENOTYPIC PROFILES

The present invention is concerned with the field of 'genetic pharmacology'. Specifically, it relates to methods which can determine, among other things, whether a compound has potential pharmacological activity, whether a compound interacts with a particular gene or biochemical pathway in man or animals, what side effects are likely to be associated with a particular pharmaceutical compound and/or the mode or modes of action of any compound with biological activity. Additional uses for the methods of the invention include the assignment of function to particular genes or assignment of genes and their encoded proteins to particular biochemical pathways. In particular, the invention relates to the use of a microscopic nematode worm, for example *Caenorhabditis elegans*, and libraries of such worms in the aforementioned methods. These new methods are able to enhance and accelerate the drug discovery process.

Prior to the early 1990's the search for new compounds having the potential to combat human or animal disease was often begun by taking a compound known to have a particular pharmacological activity, synthesising structurally related variants and then testing those variants against the known target.

The test against the target might be carried out *in vivo*, for example by use of animal models of a human disease. Alternatively, if a particular molecule was known to be implicated in the progress of a disease, the compounds could be tested for interaction with the molecule *in vitro*. The limitations of such methods are that in the event of a negative result no other information about the pharmaceutical potential of the compound tested is

gained. For example, an *in vitro* test might show a compound to have no inhibitory action against a particular target enzyme but that compound might have an inhibitory action against another enzyme in the same biochemical pathway as the target enzyme and therefore, in fact, have potential in treatment of the target disease. Animal tests, while providing a reasonable indication of both efficacy and toxicity, provide no information at all about the mode of action of the compound, and therefore the possible reasons for any toxicity. Furthermore, they are time-consuming and expensive and do not lend themselves to automation.

Since the early nineties there have been two developments in particular which have revolutionized the drug discovery process, these being the new sciences of 'genomics' and 'combinatorial chemistry'. It has now been realised that a vast number of diseases have a genetic component and they are not purely the result of environmental influences. Indeed, it is possible that nearly all diseases are multifactorial and will have some degree of genetic basis, albeit very small in some cases. A huge amount of effort is being directed at the present time to the study of the organisation of the genomes of various unicellular and multicellular organisms, including humans. This involves the identification and sequencing of all the genes in a particular genome. Such activity does not only allow for hunting of genes which are directly associated with particular diseases but each of the genes found and the proteins they encode can become, directly or indirectly, a target against which compounds can be screened, whether or not that gene has yet been associated with a disease or indeed has any identified function at all.

Furthermore, rather than starting from a compound of known 'activity' and relying on theoretical structure/function relationships to synthesise new candidate compounds, vast libraries of compounds, of uniform activity can be very rapidly synthesized in an automated manner by combinatorial chemistry. Thus, there is now potential to screen thousands of compounds against thousands of genes and the proteins they encode in very rapid high throughput screens (HTS) and to link compounds to genes and genes to disease.

The present inventors have discovered that these new technologies for drug discovery can conveniently be married with a particular multicellular organism, a nematode worm, *C.elegans,* which has been well characterised genetically and morphologically. They have thereby developed new methods, which are extremely powerful, rapid and convenient and can play an essential part in a drug discovery program.

C. elegans is a microscopic nematode worm which occurs naturally in the soil but can be easily grown in the laboratory on nutrient agar inoculated with bacteria, preferably *E. coli,* on which it feeds. Each worm grows from an embryo to an adult worm of about 1 mm long in three days or so. As it is fully transparent at all stages of its life, cell divisions, migrations and differentiation can be seen in live animals. Furthermore, although its anatomy is simple its somatic cells represent most major differentiated tissue type including muscles, neurons, intestine and epidermis. Accordingly, differences in phenotype which represent a departure from that of a wild-type worm are relatively easily observed, either directly by microscopy or by using selective staining procedures, and many of these phenotypic differences submit to quantitative measurement. Many *C. elegans* mutants have

been identified and their phenotypes described, for example, see *C. elegans* II Ed. Riddle, Blumenthal, Meyer and Priess, Cold Spring Harbor Laboratory Press, 1997. The *C. elegans* genome is now almost entirely sequenced as a result of the *C. elegans* genome project, carried out at the Sanger Center and Washington University School of Medicine. The sequence is available in a public database at http://www.sanger.ac.uk/projects/C_ elegans/. As a result of this it has emerged that *C. elegans* comprises genes which have equivalents that are widely distributed in most or all animals including humans.

Methods for creating mutant worms with mutations in selected *C. elegans* genes are known in the art, for example see J. Sutton and J. Hodgkin in 'The Nematode Caenorhabditis elegans' Ed. By William B. Wood and the Community of *C. elegans* Researchers CSHL, 1988 594-595; Zwaal et al; Target-Selected Gene Inactivation in Caenorhabditis elegans by using a Frozen Transposon Insertion Mutant Bank' 1993, Proc. Natl. Acad. Sci. USA 90 pp 7431-7435; Fire et al, Potent and Specific Genetic Interference by Double-Stranded RNA in Caenorhabditis elegans 1998, Nature 391 860-811.

The possibility that *C. elegans* might be useful for establishing links between compounds and specific *C. elegans* genes by virtue of comparison of phenotypes generated by exposure to particular compounds and by selected mutations is considered by Rand and Johnson in Methods of Cell Biology, Chapter 8, vol 84, Caenorhabditis elegans: Modern Biological Analysis of an Organism Ed. Epstein and Shakes, Academic Press, 1995 and J. Ahringer in Curr. Op. in Gen. & Dev. 7; 1997; 410-415.

However, these authors observe and attribute altered phenotypes on the basis of a single changed characteristic such as, for example, pharyngeal

pumping rate or defecation frequency. Since that single characteristic may be determined by expression of a number of genes and the operation of several biochemical pathways such a crude assessment of phenotype is not sufficient to establish a link between any one gene or pathway and a compound to which the worm has been exposed. As such the procedure would not be sensitive enough for resolution of the properties of thousands of compounds in a high throughput compound screen. An additional problem with the proposals of the prior art is that known phenotypic characteristics have all been described differently by different workers in the *C. elegans* field. Phenotype descriptions in the literature largely omit aspects not directly related to or not recognised to be related to the principle interest of the individual researcher. There is no standard nomenclature to identify a specific change. Without this it is impossible to equate newly observed phenotypes with particular known phenotypes for comparison purposes.

The present inventors have developed methods which solve these problems and thereby have converted *C. elegans* into a really useful tool in the drug discovery field. Specifically, in respect of each worm a 'phenotype profile' or 'fingerprint' is established based on looking for plurality of changed characteristics in a particular mutant or worm which has been exposed to an environmental change or a compound. Furthermore, each profile is scored by following a strict standard protocol of measurement and a standard description is applied to each characteristic. The determination of a phenotypic profile in this way for a plurality of mutants or worms exposed to compounds illuminates differences between different mutants or otherwise treated worms

which would not be apparent based on prior art methods. Furthermore, the standard scoring protocol and nomenclature allows the phenotypic profiles obtained to be collated into a library of reference profiles for direct comparison purposes. Thus, libraries of reference profiles can be established for mutant worms and for worms exposed to particular environmental changes or different sorts of compounds. Such libraries allow complex patterns of linkage to be established between particular compounds and particular genes or biochemical pathways and between individual compounds of known or unknown biochemical or pharmacological activity.

In accordance with a first aspect of the present invention there is provided a method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) providing a worm having a defect in at last one gene.

(b) measuring any changes in identifiable characteristics of said worm compared to a worm without said defect,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotype profile associated with said defect,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of worms each of which has a different defect, and

(e) collating the phenotypic profiles so obtained into a library of said profiles.

Caenorhabditis elegans is the preferred nematode worm although the method could be carried out with other nematodes and in particular with other microscopic nematodes, preferably microscopic nematodes belonging to the genus *Caenorhabditis*. As used herein the term "microscopic" nematode encompasses nematodes of approximately the same size as *C. elegans*, being of the order 1mm long in the adult stage. Microscopic nematodes of this approximate size are extremely suited for use in mid- to high-throughput screening as they can easily be grown in the wells of a multi-well plate of the type generally used in the art to perform such screening.

It is preferred to establish the phenotypic profile on the basis of the measurement and scoring of at least three different characteristics, preferably at least six characteristics and more preferably at least ten characteristics. It will be appreciated that the more differences which can be scored between a worm with a genetic defect and a worm without the defect the better the resolution between different mutants. Although not limited to such, at least one of the plurality of changed characteristics which can be measured and scored may be selected from the list shown in Table 1, and possibly each of all the changed characteristics scored is one of those shown in Table 1.

In a preferred embodiment, the method used to establish the phenotypic profile comprises measurement and scoring of two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility. This list provides a core set of measurable characteristics which can be used to establish an informative phenotypic profile for any type of worm. Furthermore,

each of these characteristics is measurable using technical measuring apparatus, such as video image analysis, multiwell plate reader, and/or a technical assay procedure. In the most preferred embodiment, the method used to establish the phenotypic profile comprises measurement and scoring of all eight of the listed core characteristics. Measuring and scoring this set of core characteristics allows meaningful comparisons to be made between phenotypic profiles for worms subjected to diverse interventions. AS exemplified herein, comparisons can be drawn between profiles for two different mutant worms and between profiles for mutant worms and profiles for worms exposed to compound.

It is to be understood the terms "measuring" or "measurement" as used in connection with any of the methods described and claimed herein are to be interpreted as including not just absolute quantitative measurement wherein a numerical value is assigned to the characteristic but also comparative measurement, wherein characteristics of a worm which has been subject to an intervention (i.e. mutation, exposure to compound, exposure to environmental change) are measured relative to the same characteristics of a wild-type worm and scored as being 'larger', 'smaller', 'longer', 'shorter', 'fatter', 'thinner', 'darker', 'paler' etc.

For comparison purposes it is essential that the scored characteristics are represented in the same order for each profile. For standardization of procedure between different workers or to facilitate automation, measurement and scoring of the characteristics could be carried out in a pre-determined order according to a standard protocol. However, this is not essential to the operation of the method. In its simplest form and as shown in Example 5, the characteristics are recorded in a binary manner

as 'present' or 'not present' based on deviations from wild-type worms.

It is desirable to establish a library which comprises a phenotypic profile in respect of a defect in each gene in the worm genome and/or different defects in the same gene (allelic variations). As aforesaid there are a considerable number of available mutants (see Riddle, Blumenthal, Meyer and Priess and Ahringer above). In addition new ones can be generated by specific gene and site directed mutation and knock-out methods known to those skilled in the art such as ethyl methanesulphonate (EMS) mutagenesis, transposon insertion or genetic interference using double stranded RNA (see Sutton and Hodgkin, Zwaal et al and Fire et al above). The known or newly generated genetic defects may manifest themselves, for example, as the absence of expression of a gene, the reduction in expression of a gene, the over-expression of a gene, the expression of a functionally defective protein, the mis-expression of a protein, the ectopic mis-expression of a protein, the expression of a protein of altered stability or the alteration of gene expression as a function of time.

Generally, the manipulation of *C. elegans* to generate genetic defects can be carried out on wild-type worms or worms with existing single or multiple mutations. It may be desirable to genetically manipulate *C. elegans* carrying a reporter gene construct. The reporter molecule might be LacZ or green fluorescent protein but many other reporter molecules are known to those skilled in the art. Reporter gene constructs for *C. elegans* are described in Chalfie et al, 1994, Science <u>263</u> pp 802-805. It can also be desirable to genetically manipulate and then profile a transgenic worm, preferably a worm carrying a human gene, particularly where the gene is

associated with, or is a candidate for association with a human disease and therefore a putative drug target. A list of human diseases for which a particular gene has been implicated is given in the paper by J. Ahringer (see above) and also provided by OMIM. Center for Medical Genetics, John Hopkins University and National Biotechnology Information, National Library of Medicine, 1996. http//www.ncbi.nlm.nih.gov/omim/, although these lists are not necessarily exhaustive.

It is easy to establish transgenic lines in *C. elegans* and the methodology is described in Craig Mello and Andrew Fire, Methods in Cell Biology, Vol 48 Ed. H.F. Epsein and D.C. Shakes, Academic Press, pages 452-480.

A form of the worm which may show a change in phenotype and may therefore be subject to profiling as described above is one in which the genetic defect and/or transgene and/or reporter gene is only present in a sub-set of the cells of the worm. It is possible for just the cells of a particular tissue to be the subject of a genetic manipulation.

The worm which is to be subject to determination of its phenotypic profile can be cultured by methods well-known in the art. *C. elegans* can grow on nutrient agar which has first been inoculated with bacteria on which the worms feed. Suitable culture methods are described in Rand and Johnson (see above) and in the examples given herein. Measurement of any changed characteristics which will determine the profile may be carried out using light microscopy, differential interference contrast optics or fluorescence microscopy. In addition immuno-chemical detection, colorimetric detection or detection of fluorescence, luminescence or radioactive labels may be used. In

some cases the changed characteristics may be biochemical only and might be detected, for example by a pH change in the growth media or a change in electrical potential. Different characteristics may need to be determined at different points in the growth cycle of the worm. For example, some phenotypic characteristics may be manifested only in the larvae while others are only detectable in the adult worm. In some cases it may be necessary to make several measurements of the same characteristic at pre-determined time intervals.

Phenotypic profiles generated by the methods described above can be collated into a library of profiles which are stored electronically on a database. However, it will be appreciated that the invention also provides a method of constructing a physical library or bank or worms each identifiable by their individual phenotypic profile. Such a worm library can be created using any or all of the methods described above and used for comparative purposes. The worms may be maintained by the culture methods described herein and/or frozen for long term storage by methods known to those skilled in the art. Libraries of phenotypic profiles or fingerprints of mutant worms or mutant worm libraries can be used to determine linkages between different genes and hence identify biochemical pathways. A particularly important use is the profiling of several mutations of the same gene and several genes of the same pathway. Different mutations in the same gene can have different phenotypes and often it is found that a careful analysis of the allelic series of a gene reveals important information that is hidden under a more severe phenotype of a null mutant (complete knock out, e.g. if it is lethal). Phenotypic profiles of different mutations of the same gene allow

characterisation of the gene by simply combining (logical OR) the profiles of all the mutations, whether they have been generated at the same time or not. It is possible, however, to handle the mutations separately and make more detailed connections, for example, concerning protein domains in case the similarity of phenotypes cluster with the sites of the mutations.

Described above are methods for constructing a library of phenotypic profiles for worms with a plurality of genetic defects or a library of mutant worms. However, in accordance with a second aspect the present invention provides a method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to a compound,

(b) measuring any changes in identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile associated with said compound,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different compounds, and

(e) collating the phenotypic profiles so obtained into a library of said profiles.

Methods for culturing *C. elegans* in the presence of a test compound are described by Rand and Johnson

mentioned above and in the examples herein. In its simplest form a solution of the compound in a suitable solvent may be spread over a bacterial lawn on an agar plate before inoculation with the worm. Additional refinements include feeding the worm with bacteria, preferably E. coli, which have taken up the compound or attaching the compound to a carrier compound which is particularly attractive to the worm.

The worms which are exposed to the compound may be wild-type worms, mutant worms, transgenic worms and/or worms carrying reporter gene constructs as already described herein. Further the measurement and scoring of a plurality of changed characteristics is carried out by exactly the same procedures as already described herein for the phenotypic profiling of mutant worms. This must be a standard format in order that direct comparisons can be made between profiles obtained on exposure to compounds and profiles exhibited by mutants.

With compound screening it is possible to build up a series of different libraries depending on the compounds being tested. For example one library can comprise profiles generated in respect of each of the known compounds in a Pharmacopoeia, in other words compounds with known pharmacological activity.

Another library can comprise profiles generated by compounds known to interact with a particular biochemical pathway, which may or may overlap with those compounds from the Pharmacopoeia. Other libraries could include profiles for known compounds but with no known biological activity or compounds which are completely new molecules such as might be generated by combinatorial chemistry. As aforesaid the present invention is not limited to the production of phenotypic profile libraries but includes libraries or banks of worms whose phenotypic profile has been altered by exposure to compounds. In particular

embodiments assays may be carried out with several concentrations of the same compound, and/or with mixtures of compounds. For example compounds from compound libraries may each be tested individually or with one or more other influencing compounds. Furthermore, such compound testing protocols may be executed against identical worms or multiple mutant and/or transgenic backgrounds. In a particular example a panel of worm strains, covering a wide range of biochemical pathways and cellular activities by means of mutations in particular pathways, as well as reporter genes, is used for testing compounds. For each compound, potentially at several concentrations, a profile is recorded for the measurable phenotypes of each of the worm strains, either in parallel or sequentially.

In a third of its aspects the invention provides a method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to an environmental change,

(b) measuring any changes in identifiable characteristics as a result of said environmental change,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotypic profile associated with said change,

(d) simultaneously or sequentially repeating steps (a) to (c) for each of a plurality of different environmental changes, and

(e) collating the phenotype profiles so obtained into a library of said profiles.

The environmental change may be, for example, a change in pH, osmolarity, temperature, exposure to radiation or exposure to bacteria or viruses. Each of these external influences may result in the manifestation of a different phenotypic profile of characteristics so that libraries of said profiles and affected worms can be constructed. Again, measurements and scoring of the profile should follow a standard protocol in order that valid comparisons can be made between these profiles and those in mutant and compound libraries.

The construction of worm and phenotypic profile libraries by the methods described above using the novel phenotypic profiling method described herein provides a very powerful tool for the discovery of new drugs. Profiles in each of the different libraries can be compared and links established between *C. elegans* genes and pathways, compounds and environmental effects. Preferably, the process of measuring and scoring the changed characteristics which go to make up the phenotypic profile is automated, making use of technical measuring apparatus. The profiles so generated may advantageously be stored electronically. Libraries of profiles can then be searched by computer which can identify identical or similar profiles, either within or between the different libraries. Quantitative data calculations, optionally in combination with boolean operations can be used.

A comparison of the profile generated by a particular compound with the profiles of particular mutants may indicate the likely gene or biochemical pathway with which the compound interacts in the worm. Other databases can then be searched for a match of

the worm gene with an equivalent human gene. The human gene might already be associated with a human disease as could be determined for example, from the OMIM database mentioned above. Thus, by use of the worm screen a potential candidate drug can be identified. The discovery of the mode of action of a compound with known pharmacological or biochemical activity is facilitated by comparing its phenotypic profile in the worm with the mutant library or environmental change library of profiles to identify possible targets for the compound. other possibilities include finding a new potential medical indication of a known compound, a medical indication for a novel compound, an alternative method of treatment of a known disease or an indication of the reason for the side effect exhibited by some known pharmaceuticals. Testing worms with compounds, scoring the phenotypic profile in the novel manner described herein and then searching previously established libraries of profiles can potentially achieve all those goals. Once a compound has been identified as having the potential to be a therapeutic agent it can be processed through the more traditional drug discovery routes. The compound can be tested in more specific in vitro tests based on the new knowledge of the target for the compound and in animal models of the target disease. Structural variants then can be generated by medicinal chemistry with a view to improving activity.

The invention will now be described with reference to the following Examples, together with accompanying Figures, in which:

Figure 1 is a schematic diagram of the left lateral view of the body of *C. elegans*. The body of *C. elegans* is divided into a head, a body and a tail region. The head region stops at the end of the

pharynx, the body stops at the rectum and the tail includes the tail whipe. *C. elegans* usually crawl on the right side. The ventral located vulva defines the ventral side of *C. elegans*.

Figure 2 is a schematic diagram of *C. elegans* showing the characteristics "hypertrophy of the head and "extensions on head".

Example 1

General Profiling by Plate Drop Assay

4ml NGM agar (see 'The Nematode Caenorhabditis Elegans' Ed. by William B. Wood and the Community of C. elegans Researchers CSHL, 1988, pg 589) is poured into 3cm plate, and seeded with approximately 5µl of an *E. coli* overnight culture and grown preferably for one week at room temperature. If a compound is to be profiled 10µl of compound dissolved in DMSO or other appropriate solution is pipetted onto the bacterial lawn. The lawn should be covered completely. (This step can be omitted if a mutant, transgenic or other worm is being profiled without compound). After overnight soaking in of compound one C. elegans (L4 stage) per plate is put in the bacterial lawn. Worms are checked after some hours, plates are incubated at 21°C and worms screened for phenotypes (control have L1 progeny growing). Plates are checked again after 4 days for phenotypes of F1 progeny (control shows all stages up to gravid hermaphrodites). Plates which have to be looked at again on subsequent days because of slow growth or for further checks are put aside. A plate protocol sheet such as that shown in Table 2 is completed deciding on one of the following routes: no effect/unspecific effect/needs to be applied at lower concentrations/needs to be profiled. If concentrations are appropriate and a decision can be made scoring of

characteristics to produce a profile can be started using the profiling list in Table 1. Because the compound is pipetted onto a bacterial lawn rather than it being incorporated into the agar, as has been done in the prior art, this method is designated a 'plate drop assay'.

Table 1

1. Compound specific phenotypes

Phenotype									Comment
1.1 Disappeared									
1.2 Determining compound action									
1.2.1 acute death without tracks									
1.2.2 acute death with tracks									
1.2.3 burst									
1.2.4 dissolving									
1.2.5 pale									
1.3 Compound response									
1.3.1 tracks not in center									
1.3.2 tracks inside									
1.3.3 tracks more outside									
1.3.4 tracks only outside									
1.3.5 tracks invisible									
1.3.6 attraction									
1.3.7 avoidance (try to avoid)									
1.3.8 avoidance (try to escape)									
1.4 Course of compound response									
1.4.1 immediate response									
1.4.2 delayed response									
1.4.3 progression of phenotype									
1.4.4 shift of phenotype									
1.4.5 recovered from exposure									
1.4.5.1 compound inactive									
1.4.5.2 irreversible									
1.4.5.3 adapted to compound									
1.5 Later exposed worm different									
1.5.1 weaker									
1.5.2 worse									
1.5.3 lower penetrance									
1.5.4 higher penetrance									
1.5.5 not affected									

2. Viability

Phenotype									Comment
Abnormal									
2.1 Dead adult (P0; during 3 days)									
2.2. Partial lethality									
2.2.1 Few dead eggs									
2.2.2 Few dead larvae									

2.3 Embryonic arrest of F1									
2.3.1 Leakyness									
2.3.2 Appearance of eggs									
2.3.2.1 dark eggs									
2.3.2.2 bright eggs									
2.3.2.3 two-fold or older									
2.3.2.4 irregular egg size									
2.4 Larval arrest of F1									
2.4.1 Leakyness									
2.4.2 at L1									
2.4.3 at L2									
2.4.4 at L3									
2.4.5 at L4									
2.5 Embryonic arrest of F2									
2.5.1 Leakyness									
2.5.2 Appearance of eggs									
2.5.2.1 irregular egg size									
2.6 Larval arrest of F2									
2.6.1 Leakyness									
2.6.2 at L1									
2.7 Died during adulthood (F1)									
2.8 Died during adulthood (F2)									

3. Life cycle

Phenotype									Comment
Abnormal									
3.1 Growth abnormal									
3.1.1 only generation cycle slowed down									
3.1.1.1 oldest stage L1									
3.1.1.2 oldest stage L2									
3.1.1.3 oldest stage L3									
3.1.1.4 oldest stage L4									
3.1.2 generation cycle slowed down while displaying defects									
3.1.2.1 oldest stage L1									
3.1.2.2 oldest stage L2									
3.1.2.3 oldest stage L3									
3.1.2.4 oldest stage L4									
3.1.3 stage changed									
3.1.3.1 delayed hatching									
3.1.3.2 arrested growth in L1									
3.2 Dauer formation defective									
3.2.1 constitutive dauer									
3.2.2 non-conditional constitutive									
3.2.3 defective									
3.2.4 dies on recovery									
3.3 Life span changed									
3.3.1 Life span is shorter									
3.3.2 Life span is prolonged									

4. Body shape

Phenotype									Comment
Abnormal									
4.1 Proportion abnormal									
4.1.1 short									
4.1.2 long									
4.1.3 thin									
4.1.4 thick									
4.1.5 small (short and thin)									
4.1.6 large (long and thick)									
4.1.7 dumpy									
4.1.7.1 piggy									
4.1.7.2 lumpy									
4.1.7.3 weak (dumpyish)									
4.1.7.4 medium									
4.1.7.5 strong									
4.2 Head defects									
4.2.1 extensions, protrusions									
4.2.2 hypertrophy									
4.2.2.1 hypertrophy ventral side									
4.2.2.2 hypertrophy dorsal side									
4.2.2.3 hypertrophy left side									
4.2.2.4 hypertrophy right side									
4.2.3 dystrophy									
4.2.3.1 dystrophy ventral side									
4.2.3.2 dystrophy dorsal side									
4.2.3.3 dystrophy left side									
4.2.3.4 dystrophy right side									
4.2.4 only head bent									
4.2.5 hammer head									
4.2.6 swollen									
4.2.7 rounded									
4.2.8 short and rounded									
4.2.9 tapering									
4.2.10 notched									
4.2.11 vacuoles only in head									
4.2.12 autodecapitation									
4.3 Body defects									
4.3.1 bent body									
4.3.2 U-shaped									
4.3.3 humpback (dorsal lumps)									
4.3.4 truncated									
4.3.5 withered									
4.3.6 twisted									
4.3.7 spindle-shaped									
4.3.8 scrawny									
4.3.9 fat									
4.3.10 pale									
4.3.11 pale with dark spots									
4.3.12 clear									
4.3.13 extensions, protrusions									
4.3.14 fluid-filled									
4.3.15 full of vacuoles									
4.4 Tail defects									
4.4.1 only tail truncated									
4.4.2 knob-like									
4.4.3 tapering									
4.4.4 only tail withered									

4.5 Cuticle defects									
4.5.1 blistered									
4.5.1.1 symmetrically									
4.5.1.2 around the head									
4.5.1.3 around the pharynx									
4.5.1.4 around the body									
4.5.1.5 around the tail									
4.5.2 moulting defective									
4.5.2.1 incomplete molts									
4.5.2.2 supernumerary molts									
4.5.3 burst									
4.6 Poured out									

5. Movement

Phenotype									Comment
Abnormal									
5.1 No movement/Motionless									
5.1.1 stiff rods									
5.1.2 loose rods									
5.1.3 lay still									
5.1.4 completely stretched out									
5.1.5 clenched									
5.1.6 jerky									
5.1.7 wiggle									
5.1.8 omega appearance									
5.1.9 capital omega appearance									
5.1.10 straight but head motion									
5.1.10.1 sniffling									
5.1.10.2 reduced head motion									
5.1.11 coiler									
5.1.11.1 tends to coil									
5.1.11.2 weak coiler									
5.1.11.3 strong coiler									
5.1.11.4 vulva always outside									
5.1.11.5 vulva always inside									
5.1.11.6 simultaneously folding in both the anterior & the posterior parts									
5.1.11.7 spiralling inwards anteriorly									
5.1.11.8 spiralling inwards posteriorly									
5.2 Slow movement									
5.3 Enhanced movement									
5.4 Irregular movement									
5.4.1 shaker									
5.4.2 erratic									
5.4.3 curly									
5.4.4 jerky movement									
5.4.5 weak kinker									
5.4.6 strong kinker									
5.4.7 preferred direction									
5.4.7.1 moves better forward									
5.4.7.2 moves better backward									
5.4.7.3 moves always forward									
5.4.7.4 moves more often backward									

5.4.8 loopy movement									
5.4.9 rolling									
5.4.9.1 right-handed									
5.4.9.2 left-handed									
5.4.10 spinning round									
5.4.10.1 in a circle									
5.4.10.2 in a curled circle									
5.4.11 kicker									
5.4.12 twitcher									
5.4.13 amplitude increased									
5.4.14 amplitude decreased									
5.4.15 amplitude weak exhibited									
5.4.16 body is dragged by head									
5.5 Head movement abnormal									
5.5.1 loopy head movement									
5.5.2 head movement reduced									
5.5.3 head movement enhanced									
5.6 Tail movement abnormal									
5.6.1 clenched									
5.6.2 tail is dragged by body									

6. Mechanotransduction (Touch with a wire and with eyelash)

Phenotype								Comment
6.1 Harsh touch response abnormal								
6.1.1 no plate drop response								
6.1.2 no movement								
6.1.3 irregular movement								
6.1.3.1 moves not forward								
6.1.3.2 moves forward abnormal								
6.1.3.3 moves not backward								
6.1.3.4 moves backward abnormal								
6.1.3.5 moves better forward								
6.1.3.6 moves better backward								
6.1.4 cramped before movement								
6.1.5 shrinker before movement								
6.2 Harsh touch reflex abnormal								
6.2.1 no plate drop reflex								
6.2.2 movement after prodding								
6.2.2.1 sleepy								
6.2.3 no reflex								
6.2.4 irregular reflex								
6.2.4.1 no move back reflex								
6.2.4.2 weak move back after reflex								
6.2.4.3 no move forward reflex								
6.2.4.4 weak move forward reflex								
6.2.5 cramped								
6.2.6 shrinker								
6.3 Nose touch avoidance abnormal								
6.3.1								
6.4 Foraging behaviour abnormal								
6.4.1								
6.5 Body touch response abnormal								
6.5.1								

7. Sensory system

Phenotype									Comment
Abnormal									
7.1 Av idance of bacteria									
7.2 Bordering behaviour									
7.3 Chemotaxis defective									
7.3.1 attraction									
7.3.2 avoidance									
7.4 Thermotaxis defective									
7.4.1 attraction									
7.4.2 avoidance									

8. Environmental response

Phenotype									Comment
Abnormal									
8.1 Osmolarity sensitive									
8.2 Thermotolerance changed									
8.3 UV Resistance changed									
8.4 Oxygen sensitive									

9. Pharynx

Phenotype									Comment
Abnormal									
9.1 Pharynx stuffed									
9.2 Morphology defects									
9.3 Pumping defects									
9.3.1 pumping reduced									
9.3.2 pumping enhanced									
9.3.3 pumping irregular									
9.3.4 no pumping									
9.4 Eating defective									

10. Intestine

Phenotype									Comment
Abnormal									
10.1 Morphology defects									
10.1.1 enlarged									
10.1.2 detached									
10.2 Color of contents									
10.2.1 darker									
10.2.2 lighter									

11. Rectum

Phenotype									Comment
Abnormal									
11.1 Morphology defects									
11.1.1 protruding									
11.1.2 scarring									
11.1.3 absent									
11.2 Constipation									
11.2.1 foregut filled/enlarged									
11.2.2 hindgut weak									
11.2.3 hindgut strong									
11.3 Defecation cycle defective									
11.3.1 expulsion defective									
11.3.1.1 weak expulsion									
11.3.1.2 no expulsion									
11.3.2 aBoc defective									
11.3.3 pBoc defective									
11.3.4 wrong timing of cycle									

12. Gonad

Phenotype								Comment
Abnormal								
12.1 Morphology defects								
12.1.1 defective gonad								
12.1.2 one arm missing								
12.1.3 multiple gonad								
12.1.4 monopolar gonad forward								
12.1.5 monopolar gonad backward								
12.1.6 no gonad								
12.2 Light brown								

13. Vulva

Phenotype									Comment
Abnormal									
13.1 Morphology defects									
13.1.1 defective vulva									
13.1.2 protruding vulva									
13.1.3 multi vulva (number)									
13.1.4 no vulva									
13.1.5 leaky vulva									
13.1.6									
13.1.7									

14. Fertility

Phenotype									Comment
Abnormal									
14.1 Brood size abnormal									
14.1.1 smaller									
14.1.2 larger									
14.2 Egg laying defect									
14.2.1 no egg retention									
14.2.2 immediate Egl									
14.2.3 progressive Egl									

14.2.4 egg laying defective									
14.2.4.1 weak Egl									
14.2.4.2 strong Egl									
14.2.5 bloated worms									
14.2.5.1 weak bloating									
14.2.5.2 strong bloating									
14.2.5.3 bags of worms									
14.2.6 no egg laying									
14.3 Only oocytes									
14.4 Sterile									
14.5 Maternal effect sterile									

15. Male

Phenotype							Comment
Abnormal							
15.1 Frequency							
15.1.1 high incidence of males							
15.2 Mating defective							
15.3 Morphology							
15.3.1 leptoderan tail							
15.3.2 scrawny							
15.3.3 copulatory plug							
15.4 Mating behaviour							
15.4.1 defective sensory contact							
15.4.1.1 no response to dorsal contact							
15.4.1.2 no response to ventral contact							
15.4.2 defective backing							
15.4.2.1 no backing							
15.4.2.2 no continued backing							
15.4.3 defective turning							
15.4.3.1 loose turns							
15.4.3.2 stop at the tail							
15.4.3.3 slide off the tail							
15.4.4 defective vulval location							
15.4.5 defective spicule insertion							

16. Progression of phenotype

Phenotype								Comment
Abnormal								
16.1 Dependent on generation								
16.1.1 F1 different from P0								
16.1.1.1 weaker								
16.1.1.2 worse								
16.1.1.3 lower penetrance								
16.1.1.4 higher penetrance								
16.1.1.5 not affected								
16.1.2 F1 different from F2								
16.2 Dependent on stage								
16.2.1 appearance of phenotype								
16.2.1.1 after L2								
16.2.1.2 during adulthood								
16.2.2 shift of phenotype								
16.3 Dependent on age								
16.3.1 phenotype gets worse								
16.3.2 phenotype gets better								

Table 2

plate	well	by	date
negative control	positive control	finished	confirmed (≥3 worms)
no effect	unspecific effect	needs to be applied at lower concentrations	needs to be profiled

Day 0

compound
invisible
coloured
droplets
crystals
complete crust

bacteria
normal lawn
grown as ring
thin
crust
died

worm
happy
run away
irregular movement
slow movement
no movement

Day 1

appearance
healthy
slightly unhealthy
slightly starved
strong starved
very sick

worm gone
lost
suicide
in agar
starved outside
died in compound

replaced by
number and stage
left progeny

movement
normal
tracks more outside
tracks not in center
amplitude increased loopy
amplitude variable
amplitude decreased
enhanced movement
slow movement
no movement
specific

body
normal gravid adult
pumping defects
light brown messy gonad
pale with dark spots
few eggs in gonad
pharynx stuffed
foregut filled large
hindgut constipated
protruding vulva
other:

progeny
normal
reduced broodsize
younger staged
oocytes
coagulated eggs
dead eggs
dying hatchlings
crippled larvae

Day 4

food
still plenty of
already finished
finished soon
outside comp.
not eatable, died

adult viability
still fertile
laying oocytes
died
died as bag of worms
missing

growth rate
normal
reduced broodsize
younger staged

movement
normal
population more outside
population not in center
amplitude increase, loopy
amplitude variable
amplitude decreased
enhanced movement
slow movement
no movement
specific:

body
normal gravid adult
pumping defects
light brown messy gonad
pale with dark spots
few eggs in gonad
pharynx stuffed
foregut filled large
hindgut constipated
protruding vulva
other:

brood viability
dead eggs
dead larvae
larval arrest
later scoring
day of screen
day of worm

comparison of phenotypes

progeny shows PC phenotype
similar
worse
a few only
weaker
no effect

comparison to other plates

new worms show phenotype
similar
worse
not all
weaker
not effect

comparison to known drugs

stage & age
all stages
young only
late larvae and adults
adults only
old adults

comparison to known mutants

Example 2

Profiling of a compound library (new compounds)

To profile new compounds from a library, the general profiling protocol is followed with the variations. Compounds are profiled once in undiluted concentration, the actual concentration being dependent on the compound library in question but will be between 0.01 mg and 1 mg of compound/10µl DMSO.

For compounds with a MW of 500 this calculates to 2-200 mM stock. Dilution in 4ml agar would be at 5-500 µM. The high dose may create lots of unspecific effect problems e.g. bacterial death and worm starvation. Thus, if necessary the compounds are applied in a second round at lower concentrations which are dilutions in DMSO of 1/3, 1/10 and 1/30 of the undiluted concentration. A concentration is finally chosen for each compound which will allow a phenotype profile to be established according to the standard procedure.

Example 3

Profiling of known compounds (biotools, pharmacopoeia)

To profile known compounds from a library the general profiling protocol is followed with the following variations. The stock solution is preferred as 100mM in DMSO and the experiment is started *ab initio* with a concentration series. The concentration series is used as described below. In one series of concentrations 15 or so worms (for a reasonable number of short term effects) are placed in the agar. In three series 1 worm each is placed on the agar to score a reasonable number of progeny. Lost worms of the latter three series of concentrations can be replaced from the

large pool where worms have been exposed to the compound in the same way. The following concentrations can be used:

conc.in 10µl drop	100mM	30mM	10mM	3 mM	1mM	0.3mM
conc.in 4ml drop	100µM	300µM	100µM	30µM	10µM	3 µM

Example 4
Comparison of agar assay to drop assay

A set of compounds from the pharmacopoeia have been profiled using the general protocol (all compounds were of known activity and are described in Martindale: The Complete Drug Reference, 32nd edition, Pharmaceutical Press 1999). The plate drop assay was compared against standard of pouring compounds into the agar as described in literature which method is designated agar assay. In the drop assay as well as in the agar assay, the compounds were added to the worm in a variety of concentrations, and the survival of the worm was scored as well as the phenotypic profile induced by the compound. The lowest concentration of a compound, still resulting in the death of the nematode was designated minimal lethal dose. The maximal concentration of a compound that did not result in the death of the nematode was designated maximal nonlethal dose. The minimal concentration of a compound that still resulted in a measurable phenotype was designated minimal effective dose. The concentrations of the compounds in the agar assay were compared to the concentrations in the drop assay. From this observation one may conclude that the newly described drop assay protocol turns out to be far more efficient for most compounds. The following table lists the calculated concentration ratio needed to get the same

effect with the compound in the agar assay (in 2 ml agar) rather than the drop assay (in 4 ml agar).

Table 3:

Compound	Site	min. lethal dose	max. nonlethal dose	min. effective dose	average potency ratio
ketanserine	serotonin rec. agonist	>610			610
tamoxifen	estrogen rec. antagonist	204	304		254
fluoxetine	serotonin reuptake inh.	124	186		154
pancuronium	nicotinic antagonist			>100	100
methoxyphenylpiperazin	α-adrenorec. ligand	>48	>146	72	88
naloxone	opioid antagonist		>44	78	60
diheptylbipyridinium	ryanodine rec. antag.	20	30	36	28
W7	calmoduline antag.	20		10	14
thapsigargin	serca antagonist				14
physostigmine	cholinesterase inh.			8	8
lobeline	nicotinic rec. ligand			4	4
riluzole	glutamase release inh.	2	2	4	2
levamisole	acetylch. rec. antag			½	½
nicotine	acetylch. rec. antag			½	½

Minimal lethal dose: rate between the lowest concentration in which the compound is lethal to the worm in both assays Maximal non-lethal dose: rate between the highest concentration in which the compound is not lethal in both assays
Minimal effective dose: rate between the lowest concentration in which the compounds results in a phenotype in both assays
Average: average of the rates

Example 5

Preferred set of informative characteristics

Worms exposed to a compound, carrying a mutation or are transgenic are examined for the following 8 informative features/phenotypes:

1. Viability

Worms are examined for viability at all stages of the life cycle, being embryogenesis, larval stages 1 to 4 and adulthood. Dead embryos are defined by not hatching within 24h and dead worms are defined by not moving, by lack of pharynx pumping, by sick or pale appearance and by lack of response to mechanical stimulation.

Method:

Embryonic lethality is measured by counting the amount of unhatched worms after 24 hours (Elispot, Zeiss). Counting of unhatched worms could also be automated using the FANS device, described below. Viability of larvae and adults is measured by dye uptake.

2. Life cycle

Progeny are examined for the length of the generation cycle in comparison to control progeny (of a wild-type worm). The stage of a synchronized progeny will be compared to the stage of a synchronized control progeny (N2, Bristol strain) after three days at 20°C. The developmental stages can be distinguished by vulva development, expression of stage-specific markers, such as collagen IV, body length and transparency.

Method:

Measuring the body length of a population allows determination of the actual stage in the life cycle

(For body shape measurement, see 3. Body shape). Expression of stage-specific markers can be examined using antibodies of the appropriate specificity, by way of example an antibody that recognizes an antigen on the surface of *C. elegans* L1 larvae has been described by Hemmer *et al.*, (1991) *J Cell Biol*, 115(5): 1237-47.

3. Body shape

Worm size is determined by measuring worm length and worm diameter.

<u>Method:</u>

The body length of a synchronized progeny of adult worms is compared to the body length of a synchronized control progeny (N2, Bristol strain). Measurement of body length can be achieved using a 'worm dispenser apparatus' which is commercially available from Union Biometrica, Inc, Somerville, MA, USA. This apparatus has properties analogous to flow cytometers, such as fluorescence activated cell scanning and sorting devices (FACS). Accordingly, it may be commonly referred to as a "FANS" apparatus, for fluorescence activated nematode scanning and sorting device (FANS). The FANS device enables the measurement of properties of microscopic nematodes, such as size, optical density, fluorescence, and luminescence.

Body size may also be measured via image analysis, in which case the measurements recorded may include worm diameter and deviation from the typical tube shape of a wild-type worm.

4. Movement behaviour

The measurement of movement behaviour can include measurement of the speed of movement, or of the

pattern of movement (e.g. direction) or both. A wild-type worm moves in a sinusoidal way forward and pauses or moves backward occasionally. Any deviation from this wild-type pattern of movement can be scored as a 'changed' characteristic.

Method:

An assay based on the following principles may be used to determine the speed of movement of a worm culture:

Nematode worms that are placed in liquid culture will move in such a way that they maintain a more or less even (or homogeneous) distribution throughout the culture. Nematode worms that are defective in movement will precipitate to the bottom in liquid culture. Due to this characteristic of nematode worms as result of their movement phenotype, it is possible to monitor and detect the difference between nematode worms that move and nematodes that do not move.

Advanced multi-well plate readers are able to detect sub-regions of the wells of multi-well plates. By using these plate readers it is possible to take measurements in selected areas of the surface of the wells of the multi-well plates. If the area of measurement is centralized, so that only the middle of the well is measured, a difference in nematode autofluorescence (fluorescence which occurs in the absence of any external marker molecule) can be observed in the wells containing nematodes that move normally as compared to wells containing nematodes that are defective for movement. For the wells containing the nematodes that move normally, a low level of autofluorescence will be observed, whilst a high level of autofluorescence can be observed in the wells that contain the nematodes that are defective in movement.

In an adaptation of the movement assay, autofluorescence measurements can be taken in two areas of the surface of the well, one measurement in the centre of the well, and on measurement on the edge of the well. Comparing the two measurements gives analogous results as in the case if only the centre of the well is measured but the additional measurement of the edge of the well results in an extra control and somewhat more distinct results.

As an alternative to the above-described movement assay, specialist software such as SIMI Scout (designed for movement study of an athlete) may be used to determine speed of movement, deviation from sinusoidal movement and even the overall pattern of movement of the worm.

5. Mechanotransduction

Worms are examined for response to mechanical stimulation.

Method:

When the plate on which *C. elegans* are cultured is dropped wild-type worms react by enhanced movement and enhanced overall activity. The capability of a worm to respond to a mechanical stimulus is measured by the difference in speed of movement before and after stimulation.

6. Pharynx pumping

The phenotypes "Pumping frequency reduced, Pharynx pumping irregular" etc. describe the activity of the cyclic contraction of the pharynx muscles that occurs in a feeding adult about 3 times in a second. The contraction cycle can be descried as the nearly simultaneously contraction of the corpus, anterior

isthmus, and terminal bulb, followed by relaxation.

Method:

The following pharynx pumping characteristics may be analyzed by image analysis: The frequency of pumping by counting the pharynx contraction. Pharynx contraction can be measured visibly by the opening and closing of the anterior corpus. The time of opened anterior corpus and the diameter of the opened corpus is used to measure hypercontraction, relaxation and strength of a contraction.

The following is an example of a pumping assay which allows measurement of the total efficiency of feeding of a worm, which is related to pumping:

The pumping rate of the pharynx is measured indirectly by adding a marker molecule precursor such as calcein-AM to the medium and measuring the formation of marker dye in the *C. elegans* gut. Calcein-AM is cleaved by esterases present in the *C. elegans* gut to release calcein, which is a fluorescent molecule. The pumping rate of the pharynx will determine how much medium will enter the gut of the worm, and hence how much calcein-AM will enter the gut of the worm. Therefore by measuring the accumulation of calcein in the nematode gut, detectable by fluorescence, it is possible to determine the pumping rate of the pharynx.

To perform the pharynx pumping screen with calcein-AM, a concentration of between 1 and 100μM calcein-AM is added into the medium. Preferably 5 to 10μM calcein-AM is used. Fluorescence is measured using a multi-well plate reader (Victor2, Wallac Oy, Finland) with following settings: Ex/Em = 485/530.

7. Defecation

The defecation of *C. elegans* is a recurrent event comprising of the following steps: pBoc, aBoc and expulsion. Defecation in nematodes such as C. elegans is achieved by periodically activating a defined sequence of muscle contractions. These contractions are started in the anterior body wall muscles. At the zenith of the anterior body contractions the four anal muscles also contract. The four anal or enteric muscles are the two intestinal muscles, the anal depressor and the anal sphincter. In addition to this series of muscle contractions, specific neurons are also involved in the regulation of defecation, including the motor neurons, AVL and DVB.

Method:

In order to construct a phenotypic profile, well-fed adults are typically examined after one day for constipation. The time between two pBocs is also scored.

The rate of defecation of *C. elegans* can also be quantitatively measured using an assay based on the following principles:

The rate of defecation of nematodes such as *C. elegans* can be easily measured using a marker molecule which is sensitive to pH, for example the fluorescent marker BCECF. This marker molecule can be loaded into the *C. elegans* gut in the form of the precursor BCECF-AM which itself is not fluorescent. If BCECF-AM is added to nematode culture medium in the wells of a multi-well plate the worms will take up the compound which is then cleaved by the esterases present in the *C. elegans* gut to release BCECF. BCECF fluorescence is sensitive to pH and under the relatively low pH

conditions in the gut of *C. elegans* (pH<6) the compound exhibits no or very low fluorescence. As a result of the defecation process the BCECF is expelled into the medium which has a higher pH than the *C. elegans* gut and the BCECF is therefore fluorescent. The level of BCECF fluorescence in the medium (measured using a multi-well plate reader on settings Ex/Em=485/550) is therefore an indicator of the rate of defecation of the nematodes.

8. Fertility

A wild-type adult hermaphrodite *C. elegans* lays about 8 eggs per hour.

Method:

The amount of eggs laid by 20 hermaphrodite *C. elegans* during at least 60 min is counted. The amount of eggs may be counted by simple visual inspection or using a FANS device, described above.

Example 6

Comparison of profiles within a library

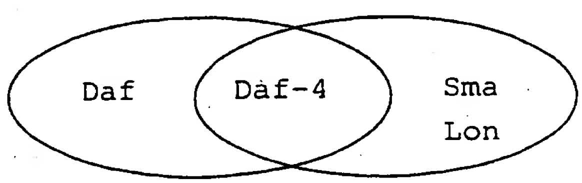
(daf-4 belongs to two pathways)

Mutant worms have been profiled according to the general profile protocol. Table 4 shows a summary of the profile, also called fingerprints, of one mutation of the indicated genes. Entries are binary with empty fields indicating a phenotype (deviation from negative control, here wild-type) not found assuming that it could have been measured. Any other entry including comments or quantitative data is read as measured phenotype in this binary scheme and indicated by *. The table lists only phenotypes that do have a

positive entry, not necessarily complete, leaving pages of empty fields alongside and arranged according to a particular enquiry. The upper half consists of the hierarchical categories "dauer formation phenotypes" and "body shape phenotypes" as well as their relevant sub-phenotypes. The lower part consists of a set of hierarchically unrelated phenotypes subsumed under the enquiry categories, "increased activity" and "decreased activity". The complete list of characteristics is to be found in Table 1.

The point of including the lower part is to show the principle of recording all observed phenotypes, that they can be used to distinguish similar phenotypic profiles in detail and that they can be arranged in order to make comparisons. In this case it is seen that the dichotomy of long versus short body length does not correlate to the dichotomy of increased versus decreased activity.

The upper part shows 5 genes (i.e. a mutation in that gene) affecting dauer formation as well as 5 genes affecting body shape in a particular combination. A mutation in one gene, daf-4, is unique in sharing the characteristics of both phenotypic groups. The following picture illustrates the phenotypic overlap as found by comparing entries in the phenotypic profiles.



From this overlap a hypothesis of a mechanistic link can be put forward for daf-4. In this particular case the mechanistic link is confirmed by the molecular

nature of the genes, which as far as known are all members of the TGFβ pathway by sequence similarity:

daf-7 TGFβ like ligand
daf-1 type I receptor
daf-4 type II receptor
daf-3 SMAD
daf-14 SMAD

dbl-1 TGFβ like ligand
sma-6 type I receptor
daf-4 type II receptor
sma-2 SMAD
sma-3 SMAD
sma-4 SMAD

The DAF-4 protein probably acts as a type II receptor in both pathways. The similarity of phenotypic profiles allows one to hypothesize mechanistic relationships in a manner analogous to sequence similarity of genes. For example a compound which induces the phenotypes: longer or shorter body length in combination with 2 or 3 of pale, thin and variable egg size, in worms exposed to it, is very likely to act on a protein of the TGFβ pathway.

Table 4:

Phenotype	*daf-1*	*daf-7*	*daf-3*	*daf-14*	*daf-4 e1364*	*sma-2 e502*	*sma-3 e491*	*sma-4 e729*	*lon-1 e185*	*lon-3 e2175*
dauer formation	●	●	●	●	●					
constitutive dauer	•	•	•	•	•					
recovery defective	•	•	•	•	•					
body shape					●	●	●	●	●	●
short					•	•	•	•		
long									•	•
thin					•	•	•	•	•	•
pale					•	•	•	•	•	

Phenotype	*daf-1*	*daf-7*	*daf-3*	*daf-14*	*daf-4 e1364*	*sma-2 e502*	*sma-3 e491*	*sma-4 e729*	*lon-1 e185*	*lon-3 e2175*
irr gular egg size					•	•		•	•	•
increased activity					●		●	●	●	●
enhanced movement					•		•		•	
amplitude increased									•	
head movement enhanced							•	•	•	•
foraging behaviour increased					•			•		•
pharynx pumping enhanced							•		•	
constitutive pumping							•	•	•	
no egg retention									•	•
decreased activity						●				
lay still						•				
slow movement						•				
pharyngeal pumping reduced						•				

Example 7

Comparison of phenotypes induced by acetylcholine esterase inhibitors

Wild type *C. elegans* adults have been exposed to acetylcholine esterase inhibitors at various concentrations. The worms have been profiled over two generations, meaning four profiles have been generated. All phenotypes from the phenotype list are displayed that have been measured in this experiment. Two phenotypes "loopy head movement" and "body dragged by head" are shared by most of the esterase inhibitors. This is called phenotype activity

relationship (PAR, by analogy to structure activity relationship SAR). The shared phenotypes are used to identify the action of a new compound. The unshared phenotypes are used to distinguish drugs or unravel side effects when these phenotypes are part of another PAR.

Table 5:

Phenotypes	Physostigmine	Neostigmine	Ambenonium	Tacrine	Galantamine	Trichlorfon
Thin	X					
Lay still	X					
Erratic	X					
Weak kinker		X				
Jerky				X		X
Enhanced head movement						X
Loopy head movement	X	X		X(L1)		X
Body dragged by head	X	X				X
Irregular touch response	X	X				
Reduced brood size	(X)					X
Delayed growth						X

Example 8

Comparison of phenotypes of mutations in the acetylcholine neurotransmission pathway

C. elegans adults and larval stages that are homozygous for the mutations *cha-1*, *unc-17*, *snt-1* and *cat-1* have been profiled, meaning fingerprints have been generated. All phenotypes from the phenotype list are displayed that have been scored in this

experiment. The phenotypes "small", "resistance to CHA inhibitors (Ric)", "slow pumping" and "slow growth" are shared. This is called phenotype activity relationship (PAR, in analogy to structure activity relationship SAR). The shared phenotypes are used to identify genes in a pathway. The unshared phenotypes are used to distinguish these genes or unravel further functions in parallel or new pathways when these phenotypes are part of another PAR. The fingerprint of *cat-1* is different because this gene is involved in the dopamine pathway.

Table 6:

Phenotype	*cha-1* ChAT (synthesis)	*unc-17* VchAT (ACh-transporter)	*snt-1=ric-2* Synaptotagmin homolog	*cat-1* VMAT (monamine-transporter)
Coiler	X	X		
Small	X	X	X	
Slow growth	X	X	X	
Ric	X	X	X	
Slow pumping	X	X	X	
Jerky when backing	X			
Low ChAT level	X			
Poor male turning				X
Enhanced foraging behaviour				
Enhanced foraging behaviour				X
Defecation defects				X
Shrinker-uncs				

Example 9

Method to profile an intervention (mutation, compound etc)

Profiling a mutation in the gene *unc-17* that affects transportation of acetylcholine.

In the literature this phenotype is described, concerning movement, body size and feeding, as severe coiler, being rather small and thin and has only slow, irregular pumping of the pharynx (Riddle et al., "C. elegans II" Cold Spring Harbor Laboratory Press, 1997). By systematically describing *unc-17* the resulting fingerprint unravels more details and new properties: Concerning movement, body size and feeding the phenotypes strong coiler, spiralling inwards posteriorly, curly jerky and moves better forward, being small have been profiled. In addition defects in the sensory system, defecation and reproductive system have been found, in detail: the touch response is gone, constipation, aberrant defecation cycle (aBoc) and egg laying defective (no egg retention).

Example 10

Method to add biological information to a particular phenotype

One phenotype of the mutation *unc-4* is "coiler" (looks like a snail). The fingerprint of *unc-4* adds for "coiler" the details "ventral side out" and "spiralling inwards posteriorly". This occurs when a set of neurons that control the forward movement of the ventral part of the worm (VA2 - VA10) gets the same input than another set of neurons that controls the backward movement of the ventral part (VB2 -

VB10).

In this case the ventral muscles get contradicting signals and only the dorsal muscles contract properly. The result is a coiler that has only the ventral side outwards. We explain most of the phenotypes as consequence of a mislead process, here synaptic input.

Example 11

Comparison of phenotypes induced by compounds acting on GABAnergic neurotransmission

Wild-type *C. elegans* adults have been exposed to GABA agonists (Muscimol) and GABA antagonists (Ivermectin and Fipronil) at various concentrations. Worms have been profiled and the scored phenotypes are displayed as fingerprints.

In addition, two mutations in the GABAnergic pathway have been profiled and compared with the compound induced phenotypes: *unc-25* encodes for the decarboxylase and *unc-49* encodes for a GABA receptor.

The phenotype "shrinker" is present in all fingerprints (see Table dark grey). This phenotype is used as marker or diagnostic phenotype to identify activity of a compound or gene in the GABAnergic pathway. There are further phenotypes only shared by some compounds and mutants (see Table light grey). These phenotypes are used to build a phenotype activity relationship (PAR).

The shared phenotypes are used to identify the action of a new compound when "shrinker" cannot be used or to reveal more details on a compound action. For example,

all compounds and *unc-25* fingerprints contain constipation phenotypes but not the fingerprint of *unc-49*, although GABA is used for the defecation process. This is coincident with earlier findings that the UNC-49 gene product is not required for defecation.

These results may indicate the existence of another yet unknown GABA receptor in *C. elegans*. The unshared phenotypes are used to unravel toxic side effects or other mode of actions.

Table 7:

Phenotypes	Muscimol	Ivermectin	Fipronil	*unc-25*	*unc-49*
Pale	x	x		X	
Motionless (paralyzed) I	x	x			
Nearly motionless	x	x			
No movement but motion II	x		x	X	x
Little movement	x		x	X	x
Slow movement III	x		x	X	
Enhanced movement V					
Stiff rods					
Loose rods	x	x			
Rigid paralysis (hypercontracted)					
Flaccid paralysis (relaxed)	x	x			
Bent body, jerky body, abnormal			x	(x)	
Omega appearance			x		x
Enhanced foraging				X	
Shrinker before movement	x		x		
Shrinker	X	X	X	X	X
No pumping	x	x			
Weak pumping					
Pumping frequency reduced		x	x		
Pumping frequency enhanced					
Pumping irregular	x				
Constipation		x	x	X	
Foregut filled/enlarged			x		
Hindgut weak constipated		x	x	X	
Hindgut strong constipated				X	
Defecation cycle defective	x	x	x	x	
(time: pBoc)					
Weak expulsion				x	
N expulsion				x	
N gg retention (12-cell stag)					
W ak egg laying defect (comma)					
Strong egg laying defect (pr tzel)		x	x		
Bloated worms			x		
Bags of worms			x		

Example 12

Definition of body shape phenotypes

Aberrations of the body shape of *C. elegans* can be the result of mutations in a vast amount of genes. These genes may be required directly for the formation of the hypodermis, the hydroskeleton and the correct patterning of the worm body plan, e.g., collagen or even-skipped. They could be involved in the control of growth or metabolism like genes of the TGF β pathway or genes required for feeding. Eventually, mutations in certain genes that cause primary defects, e.g., absence of head muscle, cause secondary defects in the body shape like dystrophy in the head region.
Body shape phenotypes are all visible or measurable deviations of the body shape, colour and content. Phenotypes are comparatively measured against wild-type (N2, Bristol strain) and scored as deviation of wild type in the corresponding developmental stage, sex and preparation. The scored phenotype comes with the percentage of worms positive for that phenotype within a population.

Table 8: Scientific definition of body shape phenotypes. The phenotypes listed in the left column are described and defined in the right column. Some phenotypes are derived from the classical worm jargon like "dumpy", which is still shorter than "short and thick worm".

PHENOTYPE	DEFINITION
Proportion abnormal	
Short	Body length less than wild type.
Long	Body length more than wild type.
Thin	Body diameter less than wild type.
Thick	Body diameter more than wild type.

Dumpy	Body length less but body diameter more than wild type.
Spindle-shaped	body diameter is more for only a restricted region of the body.

Head defects

Hypertrophy of the head	Regions of the head are thickened. This additional tissue is part of the head and enclosed by the hypodermis.
Extensions of head	Small hypertrophied regions of the head.
Notched head	Extensions, protrusions on the dorsal side of the head.
Hammer head	Extensions at the head tip resemble a hammer like appearance.
Dystrophy of the head	Regions of the head are thinned due to missing tissue.
Swollen	The head looks like a balloon.
Rounded	The tip of the head is rounded.
Tapering	The tip of the head is tapering.
Vacuoles only in head	Vacuoles visible in the head but not in the rest of the body.
Only head bent	The head is held most of the time in a bent position. In extreme cases the worm looks like a walking stick.
Autodecapitation	The head/body connection is thinner, which results occasionally in an autodecapitation due to a body wall muscle contraction.

Body defects

Scrawny	Worm is shorter, thinner, pale and sick.
Hypertrophy of body	Regions of the body are thickened. This additional tissue is part of the body and is enclosed by the hypodermis.
Extensions	Small hypertrophied regions of the body.
Humpback	Extensions, protrusions on the dorsal side of the body. The counterpart, extensions on the ventral side of the body, would be scored as "multi vulva" in the section "Vulva". The distinction between a non vulva-like extension versus a vulva-like extension will be made with a high power microscope.
Truncated body	Part of the body is missing.
Withered body	Part of the body is thinned.
Twisted	Twisted body. The rotation along the anterior-posterior body axis can be seen by the twisted gut/gonad tube or because the vulva and the rectum are not orientated in the same (ventral) direction.
Fat	Worm is thicker and darker than wild type.
Pale	Worm is brighter than wild type.
Pal with dark spots	Worm is brighter than wild type and contains dark spots.
Clear	Worm is nearly transparent.
Full of vacuoles	Worm contains more vacu les than wild type. Vacuol s have a darker or opal appearance and resemble little moon craters.
Fluid-filled	Liquid flows all over the body.

Poured out	Contents f the w rm like the gonad is released through the vulva.
Burst	Dead worm with bursted body shape.

Tail defects

Only tail truncated	Blunt body end; whipe is missing.
Tail shape aberrant	Tail or tail whipe is kinked, shortened or thickened.
Knob-like	Tail whipe has knob-like structures.

Cuticle defects

Blistered	Fluid-filled transparent blisters separated by the hypodermis outside on the body. Clearly different from extensions.
Molting defective	More worms are caught in their old skin like the sloughing of a snake.

It is possible to score body shape phenotypes by image acquisition followed by image analysis. The advantage in the automation of the profiling procedure is the quantification of the strength of a phenotype or the presence of the phenotype in a population. A disadvantage is that the procedure for analysing an image for every possible phenotype may be more elaborate than simply scoring by eye. Furthermore, certain details are difficult to access by video analysis e.g., blister versus protrusions.

Table 10: list of scientific body shape phenotypes, together with their corresponding technical definitions , in terms of characteristics which can be comparatively measured relative to wild-type characteristics using automated measuring apparatus.

Scientific phenotype	Technical definition	Technical phenotype
Proportion abnormal		
Short	Body length less than wild type	Short
Long	Body length more than wild type	Long
Thin	Body diameter less than wild type	Thin

Thick	Body diameter more than wild type	Thick
Dumpy		*Disappears*
Spindle-shaped		*Disappears*
Head defects		
Hypertrophied head	Total head volume has increased	Hypertrophied head
Extensions on head	Head will be subdivided in n trapezes (or n slices). The diameter of different trapezes can be compared pairwise. The deviation of the diameter can also be located to one side	Extensions on head
Notched head		Extensions only on one side
Hammer head		Extensions are pairwise
Dystrophied head	Total head volume has decreased	Dystrophied head
Swollen		*Disappears*
Rounded	In the tip trapeze the top diameter is increased	Rounded
Tapering	The diameter of the tip trapezes are decreased	Tapering
Vacuoles only in head		*Disappears*
Only head bent	The head is most of the time in a certain position that can be measured by an average angle between tip and head/body connection	Tip of head is more often in one position
Autodecapitation		*Disappears*

Example 13

Use of GFP in profiling *C. elegans*

A lot of features of *C. elegans* as described in Table 1 can be easily monitored, either automatically by image analysis, microtiter plate readers, or visual means, e.g. by normal microscopy or by Nomarski microscopy. Some features of *C. elegans* are more difficult to visualize. For these characteristics transgenic animals expressing a marker gene are very useful. Moreover, even for characteristics that are rather easily to score, the use of a nematode expressing a marker gene, such as GFP, LacZ, or luciferase, enhances the fingerprinting of *C. elegans*.

The *C. elegans* can be a wild type, a mutant, or a strain subjected to a compound or environmental stress, or a combination of those.

C. elegans mutant *unc-23* has a fingerprint, which comprises "jerky movement", "tend to coil", "bent head" and "egl". Expressing GFP in the muscle cells of the animal could result in identification and scoring of additional characteristics such as "improperly folded muscles", and/or "detached muscles in head region", and/or "no muscles in head region", and/or "defective muscle attachment", and/or "vulva muscle defects" (data not shown).

Similarly, *C. elegans* mutant *unc-71* has a fingerprint which comprise "reduced movement", "weak amplitude", "strong kinker", and "slightly egl". When introducing GFP in the neurons of the animals no apparent extra fingerprint features where observed. A closer look at the neurons of this mutant worm revealed at least following extra phenotypes: "fasculation defects", "VD/DC connection defects" (data not shown).

GFP-phenotypes are hence very important in allowing phenotypes which are not otherwise visible to be measurable with Nomarski or dissection microscopy. GFP-phenotypes are further important in the pinpointing of defects to certain tissues and cells, and moreover GFP-phenotypes are important in distinguishing between similar defects with different causes.

Claims:

1. A method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) providing a worm having a defect in at least one gene,

(b) measuring any changes in identifiable characteristics of said worm compared to a worm without said defect,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotypic profile associated with said defect,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of worms each of which has a different defect, and

(e) collating the phenotypic profiles so obtained into a library of said profiles.

2. A method as claimed in claim 1 wherein in step (c) at least three changed characteristics are scored.

3. A method as claimed in claim 1 or claim 2 wherein in step (c) at least six changed characteristics are scored.

4. A method as claimed in any preceding claim wherein in step (c) at least ten characteristics are scored.

5. A method as claimed in any preceding claim wherein said worm is Caenorhabditis elegans.

6. A method as claimed in any preceding claim wherein steps (a) to (c) are carried out in respect of substantially every gene in the worm genome.

7. A method as claimed in any preceding claim which includes the step of manipulating said worm to generate said defect in said at least one gene.

8. A method as claimed in any preceding claim wherein said defect is selected from the absence of expression of said gene, the reduction in expression of said gene, the over-expression of said gene, the expression of a functionally defective protein, the expression of a truncated protein, the misexpression of a protein, the ectopic misexpression of a protein, the expression of a protein of altered stability or the alteration of gene expression as a function of time.

9. A method as claimed in claim 7 or 8 wherein said manipulation is carried out on wild-type *C. elegans* or a selected mutant thereof.

10. A method as claimed in claim 9 wherein said selected mutant harbours multiple mutations.

11. A method as claimed in claim 7 or 8 wherein said manipulation is carried out on *C. elegans* carrying a reporter gene.

12. A method as claimed in claim 11 wherein said reporter gene is LacZ or green fluorescent protein (GFP).

13. A method as claimed in any one of claims 7 to 12 wherein said manipulation is carried out on a transgenic *C. elegans*.

14. A method as claimed in claim 13 wherein said transgenic *C. elegans* expresses a human gene.

15. A method as claimed in claim 14 wherein said human gene is a known drug target.

16. A method as claimed in claim 14 or claim 15 wherein said human gene is one associated with a human disease.

17. A method as claimed in claim 14 or 15 wherein said human gene is a candidate human disease gene.

18. A method as claimed in any of claims 7 to 17 wherein said manipulation is carried out on only a sub-set of *C. elegans* cells.

19. A method as claimed in any preceding claim wherein changed characteristics in said worm carrying said defect compared to a worm that does not carry said defect are identified by light microscopy, differential interference contrast optics, fluorescence microscopy, immunochemical detection or spectrophotometric detection, radiation detection, calorimetric detection, fluorescence detection or luminescence detection.

20. A method as claimed in any preceding claim wherein changed characteristics in said worm carrying said defect compared to a worm that does not carry said defect are identified by a pH change or a change in electrical potential.

21. A method as claimed in any preceding claim wherein said plurality of changed characteristics are scored in a predetermined order to generate said phenotypic profile.

22. A method as claimed in any preceding claim wherein the scoring of said plurality of changed characteristics is repeated at predetermined intervals of time.

23. A method as claimed in any preceding claim wherein said phenotypic profiles are stored electronically.

24. A method as claimed in any preceding claim wherein at least one of said plurality of characteristics is selected from the list shown in Table 1.

25. A method as claimed in any one of the preceding claims wherein step (b) comprises measuring changes in two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility.

26. A method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to a compound,

(b) measuring any changes in identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any

said changed characteristics to establish a phenotypic profile associated with said compound,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different compounds and

(e) collating the phenotypic profiles so obtained into a library of said profiles.

27. A method as claimed in claim 26 wherein in step (c) at least three changed characteristics are scored.

28. A method as claimed in claim 27 wherein in step (c) at last six changed characteristics are scored.

29. A method as claimed in claim 28 wherein in step(c) at least ten changed characteristics are scored.

30. A method as claimed in any one of claims 26 to 29 wherein said nematode worm is *C. elegans*.

31. A method as claimed in any one of claims 26 to 30 wherein each of said plurality of different compounds has a known pharmacological activity.

32. A method as claimed in any one of claims 26 to 30 wherein each of said plurality of different compounds is one which is known to interact with a particular biochemical pathway.

33. A method as claimed in any one of claims 26 to 30 wherein each of said plurality of different compounds has no known pharmacological activity or

biochemical interaction.

34. A method as claimed in any one of claims 26 to 30 wherein each of said plurality of different compounds is from a combinatorial library.

35. A method as claimed in any one of claims 26 to 34 wherein said worm to which said compound is exposed is wild-type *C. elegans* or a selected mutant thereof.

36. A method as claimed in claim 35 wherein said selected mutant harbours multiple mutations.

37. A method as claimed in any one of claims 26 to 34 wherein said worm to which said compound is exposed is *C. elegans* carrying a reporter gene.

38. A method as claimed in claim 37 wherein said reporter gene is LacZ or GFP.

39. A method as claimed in any one of claims 26 to 38 wherein said worm to which said compound is exposed is a transgenic *C. elegans*.

40. A method as claimed in claim 39 wherein said transgenic *C. elegans* expresses a human gene.

41. A method as claimed in claim 40 wherein said human gene is a known drug target.

42. A method as claimed in claim 40 wherein said human gene is one associated with a human disease.

43. A method as claimed in claim 40 wherein said human gene is a candidate disease gene.

44. A method as claimed in any one of claims 30 to 43 wherein said worm is exposed to said compound by feeding the worm on bacteria which have been exposed to said compound.

45. A method as claimed in claim 44 wherein said bacteria are *E. coli*.

46. A method as claimed in any one of claims 26 to 45 wherein said compound is linked to another compound or carrier substance.

47. A method as claimed in anyone of claims 26 to 46 wherein any changed characteristics in said worm resulting from exposure to said compound are identified by light microscopy, differential interference contrast optics, fluorescence microscopy, immunochemical detection, spectrophotometric detection, radiation detection, colorimetric detection, fluorescence detection or luminescence detection.

48. A method as claimed in any one of claims 26 to 47 wherein any changed characteristics in said worm resulting from said compound are identified by a pH change or a change in electrical potential.

49. A method as claimed in any one of claims 26 to 48 wherein said plurality of changed characteristics are scored in a predetermined order to generate said profile.

50. A method as claimed in any one of claims 26 to 49 wherein the scoring said plurality of changed characteristics is repeated at predetermined time intervals.

51. A method as claimed in any one of claims 26 to 50 wherein said scoring of changed characteristics is carried out using essentially the same scoring protocol as used in a method in accordance with any one of claims 1 to 25.

52. A method as claimed in any one of claims 26 to 51 which comprises the further step of storing the said phenotypic profiles electronically.

53. A method as claimed in any one of claims 26 to 52 wherein at least one of said plurality of characteristics is selected from the list shown in Table 1.

54. A method as claimed in any one of claims 26 to 53 wherein step (b) comprises measuring changes in two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility.

55. A method of constructing a library of phenotypic profiles of nematode worms which comprises the steps of:

(a) exposing a worm to an environmental change,

(b) measuring any changes in identifiable characteristics as a result of said environmental change,

(c) systematically scoring a plurality of any said changed characteristics to establish a Characteristic phenotypic profile associated with said change,

(d) simultaneously or sequentially repeating steps (a) to (c) for each of a plurality of different environmental changes and (e) collating the phenotypic profiles so obtained into a library of said profiles.

56. A method as claimed in claim 55 wherein in step (c) at least three changed characteristics are scored.

57. A method as claimed in claim 56 wherein in step (c) at least six changed characteristics are scored.

58. A method as claimed in claim 57 wherein in step (c) at least ten changed characteristics are scored.

59. A method as claimed in any of claims 55 to 58 wherein said environmental change is a change in the pH to which the worm is exposed and in step (d) each of the plurality of environmental changes comprises a different pH.

60. A method as claimed in any one of claims 55 to 58 wherein said environmental change is a change in the osmolarity to which the worm is exposed and in step (d) each of the plurality of environmental changes comprises a different osmolarity.

61. A method as claimed in any one of claims 55 to 58 wherein said environmental change is a change in the temperature to which the worm is exposed and in step (d) each of the plurality of environmental changes comprises a change in temperature.

62. A method as claimed in any one of claims 55 to 58 wherein said environmental change comprises

exposure to radiation and in step (d) each of said plurality of environmental changes comprises a different level of radiation.

63. A method as claimed in any one of claims 55 to 58 wherein said environmental change comprises exposure to a virus and in step (d) each of said plurality of environmental changes comprises exposure to a different virus.

64. A method as claimed in any one of claims 55 to 58 wherein said environmental change comprises exposure to a bacterium and in step (d) each of said plurality of environmental changes comprises exposure to a different bacterium.

65. A method as claimed in any one of claims 55 to 64 wherein said worm is *C. elegans*.

66. A method as claimed in any one of claims 55 to 65 including a further feature as defined in any one of claims 5 to 54.

67. A method as claimed in any one of claims 55 to 66 wherein said scoring of changed characteristics is carried out using essentially the same scoring protocol as used in a method in accordance with claims 1 to 54.

68. A method as claimed in any one of claims 55 to 67 wherein step (b) comprises measuring changes in two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility.

69. A method of constructing a multiple library

of phenotypic profiles of nematode worms which method comprises carrying out all of the methods of claims 1, 26 and 55.

70. A method as claimed in claim 69 wherein step (b) of the method of at least one of claims 1, 26 and 55 comprises measuring changes in two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility.

71. A method of determining the mode of action of a compound which method comprises the steps of;

(a) exposing a nematode worm to said compound

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of changed characteristics to establish a phenotypic profile associated with said compound and

(d) comparing said phenotypic profile with a library of reference phenotypic profiles wherein said library of reference profiles is obtainable by carrying a method in accordance with any of claims 1 to 70.

72. A method of determining whether a compound or combination of compounds interacts with a particular gene or biochemical pathway which method comprises the steps of;

(a) exposing a nematode worm to said compound or

combination of compounds

(b) measuring any changes in identifiable characteristics of said worm as a result of said exposure,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile associated with said compound or combination of compounds, and

(d) comparing said profile with a library of reference profiles said library of reference profiles being obtainable by carrying out the method of any one of claims 1 to 70.

73. A method of finding an alternative treatment for a human disease which method comprises the steps of:

(a) exposing a nematode worm to a candidate compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by carrying out a method in accordance with claim 31.

74. A method of finding a biochemical pathway in

which a compound known to have pharmacological activity acts which method comprises the steps of:

(a) exposing a nematode worm to the known compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by carrying out a method in accordance with claim 32.

75. A method of finding a potential new medicinal indication for a compound of known pharmaceutical activity which method comprises the steps of:

(a) exposing a nematode worm to the known compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound and

(d) comparing said profile with a library of reference profiles, said library of reference profiles

being obtainable by carrying out a method in accordance with any one of claims 1 to 70.

76. A method as claimed in claim 75 wherein said library of reference profiles is obtainable by carrying out a method in accordance with any one of claims 24 to 26.

77. A method of identifying the mechanism of action of any side effects associated with a compound of known pharmaceutical activity which method comprises the steps of;

(a) exposing a nematode worm to the known compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by carrying out a method in accordance with claim 32 and/or any of claims 1 to 25.

78. A method of attributing a particular gene to a particular biochemical pathway in *C. elegans* which method comprises the steps of:

(a) exposing a nematode worm to a compound known to operate in a particular biochemical pathway,

(b) measuring any changes in the identifiable

characteristics of said worm as a result of exposure to said compound

(c) systematically scoring a plurality of any changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said, profile with a library of reference phenotypic profiles said library of reference profiles being obtainable by carrying out a method in accordance with any one of claims 1 to 25.

79. A method as claimed in any of claims 71 to 78 wherein said nematode worm is selected from wild-type *C. elegans*, a mutant C. *elegans* comprising one or more mutations, a C. *elegans* carrying a reporter gene or a transgenic *C. elegans*.

80. A method as claimed in claim 79 wherein said transgenic *C. elegans* expresses a human gene.

81. A method as claimed in any one of claims 71 to 80 wherein step (b) comprises measuring changes in two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility.

82. A method for elucidating biochemical pathways in a nematode worm which method comprises the steps of:

(a) generating a defect in at least one gene in said worm,

(b) measuring any changes in identifiable characteristics of said worm compared to a worm

without said defect,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said defect, and

(d) comparing said profile with a library of reference phenotypic profiles, said library of references profiles being obtainable by carrying out a method in accordance with any one of claims 1 to 25.

83. A method as claimed in claim 82 wherein said nematode worm is selected from wild-type *C. elegans*, a mutant *C. elegans* comprising one or more mutations, a *C. elegans* carrying a reporter gene or a transgenic *C. elegans*.

84. A method as claimed in claim 82 wherein said defect is selected from the absence of expression of said gene, the reduction in expression of said gene, the expression of a functionally defective protein, the expression of a truncated protein, the misexpression of a protein, the ectopic misexpression of a protein, the expression of a protein of altered stability or the alteration of gene expression as a function of time.

85. A method as claimed in any one of claims 82 to 84 wherein at least three, preferably at least six and more preferably at least ten changed characteristics are scored.

86. A method as claimed in any of claims 82 to 85 which includes the features described in any one of claims 19 to 25.

87. A method of constructing a library of

nematode worms which method comprises the steps of:

(a) providing a worm having a defect in at least one gene.

(b) measuring any changes in identifiable characteristics of said worm compared to a worm without said defect,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotypic profile associated with said defect,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of worms, and

(e) producing a library of said worms each identifiable by their phenotypic profiles.

88. A method as claimed in claim 87 wherein said phenotypic profiles are collated into a library.

89. A method as claimed in claim 87 and 88 comprising any one of the features described in any one of claims 2 to 25.

90. A method of constructing a library of nematode worms which method comprises the steps of:

(a) exposing a worm to a compound,

(b) measuring any changes in identifiable characteristics of said worm as a result of exposure to said compound,

(c) systemically scoring a plurality of any said changed characteristics to establish a phenotypic profile associated with said compound,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different compounds, and producing a library of said worms each identifiable by their phenotypic profiles.

91. A method as claimed in claim 90 wherein said phenotypic profiles are collated into a library.

92. A method as claimed in claim 90 or 91 comprising any one of the features disclosed in any one of claims 27 to 54.

93. A method of constructing a library of nematode worms which method comprises the steps of:

(a) exposing a worm to an environmental change,

(b) measuring any changes in identifiable characteristics as a result of said environmental change,

(c) systematically scoring a plurality of any said changed characteristics to establish a characteristic phenotypic profile associated with said change,

(d) simultaneously or sequentially repeating steps (a) to (c) in respect of each of a plurality of different environmental changes, and

(e) producing a library of said worms each identifiable by their phenotypic profile.

94. A method as claimed in claim 93 wherein said

phenotypic profiles are collated into a library.

95. A method as claimed in claim 93 or claim 94 comprising any one of the features disclosed in any one of claims 56 to 70.

96. A method of determining the mode of action of a compound which method comprises the step of:

(a) exposing a nematode worm to said compound,

(b)measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile associated with said compounds, and

(d) comparing said phenotypic profile with the library of phenotypic profiles obtainable by the method of any one of claims 88, 91 or 94.

97. A method of determining whether a compound or a combination of compounds interacts with a particular gene or biochemical pathway which method comprises the steps of:

(a) exposing an nematode worm to said compound or combination of compounds,

(b) measuring any changes in identifiable characteristics of said worm as a result of said exposure,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic

profile associated with said compounds or combination of compounds, and

(d) comparing said phenotypic profile with a library of reference profiles wherein said library of reference profiles is obtainable by the method of any one of claims 88, 91 or 94.

98. A method of finding an alternative treatment for a human disease which method comprises the steps of:

(a) exposing an nematode worm to a candidate compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of 35referenced profiles, wherein said library of referenced profiles is obtainable by carrying out the method in accordance with any one of claims 88, 91 or 94.

99. A method of finding a biochemical pathway in which a compound known to have pharmacological activity acts which method comprises the steps of:

(a) exposing a nematode worm to the known compound, measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by the method of any one of claims 88, 91 or 94.

100. A method of finding a potential new medicinal indication for a compound of known pharmaceutical activity which method comprises the steps of:

(a) exposing an nematode worm to the known compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by the method of any one of claims 88, 91 or 94.

101. A method of identifying the mechanism of action of any side effects associated with a compound of known pharmaceutical activity which method

comprises the steps of:

(a) exposing a nematode worm to the known compound,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systematically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference profiles, said library of reference profiles being obtainable by the method of any one of claims 88, 91 or 94.

102. A method of attributing a particular gene to a particular biochemical pathway in *C. elegans* which method comprises the steps of:

(a) exposing a nematode worm to a compound known to operate in a particular biochemical pathway,

(b) measuring any changes in the identifiable characteristics of said worm as a result of exposure to said compound,

(c) systemically scoring a plurality of any said changed characteristics to establish a phenotypic profile for said compound, and

(d) comparing said profile with a library of reference phenotypic profiles, said library of reference profiles being obtainable by carrying out the method in accordance with any one of claims 88, 91

or 94.

103. A method as claimed in any one of claims 96 to 102 wherein said nematode worm is selected from wild-type *C. elegans*, a mutant *C. elegans* comprising one or more mutations, a *C. elegans* carrying a reporter gene or a transgenic *C. elegans*.

104. A method as claimed in claim 103 wherein said transgenic *C. elegans* expresses a human gene.

105. A method of establishing a phenotypic profile for a nematode worm which method comprises measuring and scoring at least three, preferably at least six and more preferably at least ten characteristics of said worm which are not exhibited by wild-type worms.

106. A method as claimed in claim 105 wherein said characteristics not exhibited by wild-type worms are selected from the list shown in Table 1.

107. A method as claimed in claim 105 or claim 106 which comprises measuring and scoring changes in two or more characteristics selected from the group consisting of: viability, life cycle, body shape, movement behaviour, mechanotransduction, pharynx pumping, defecation and fertility.

108. A method as claimed in any one of claims 105 to 107 wherein said phenotypic profile is established for a nematode worm which is selected from a worm having one or more mutations, a worm which has been exposed to a compound or combination of compounds, a transgenic worm, a worm carrying a reporter gene or a worm which has been exposed to an environmental change.

109. A method as claimed in claim 108 wherein said transgenic worm comprises a human gene.

110. A method as claimed in claim 108 wherein said compound has known pharmacological activity.

111. A method as claimed in claim 108 wherein said compound is known to be active in a particular biochemical pathway.

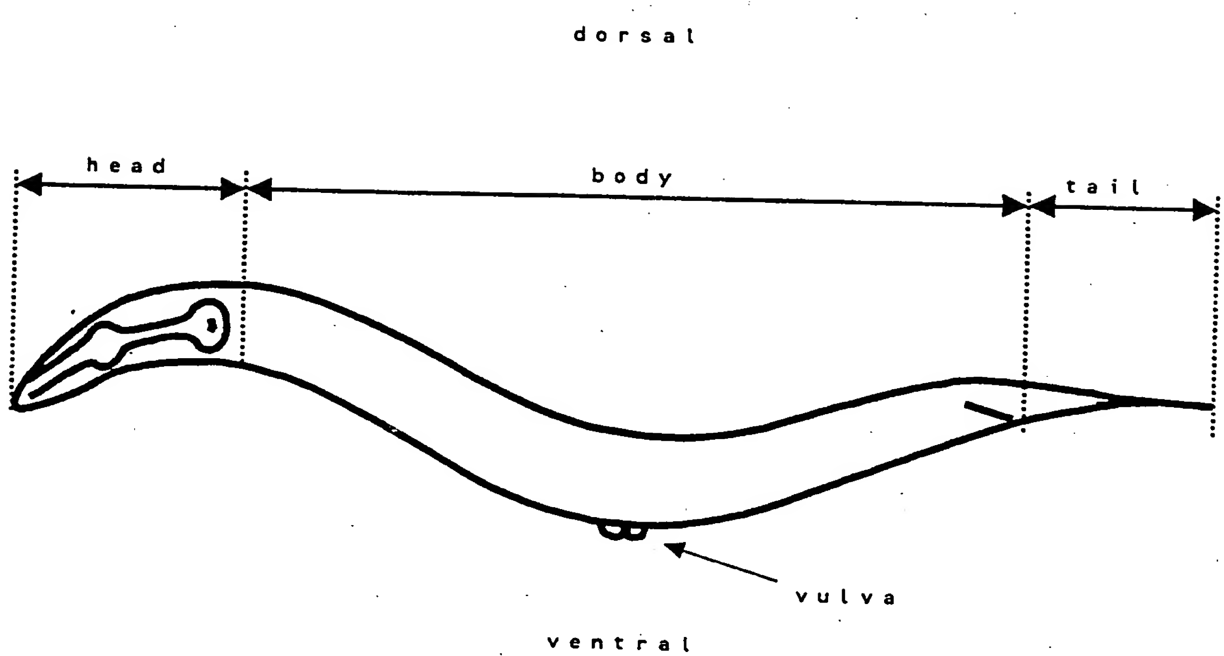
112. A method as claimed in claim 108 wherein said compound or combination of compounds is from a combinatorial library of compounds.

113. A compound which has potential therapeutic activity in a mammal which has been identified in a method as claimed in any one of claims 71 to 81 or 96 to 104.

114. A library of nematode worms obtainable by a method as claimed in any one of claims 87 to 95.

115. A library as claimed in claim 114 wherein said nematode worm is *C. elegans*.

FIG. 1.



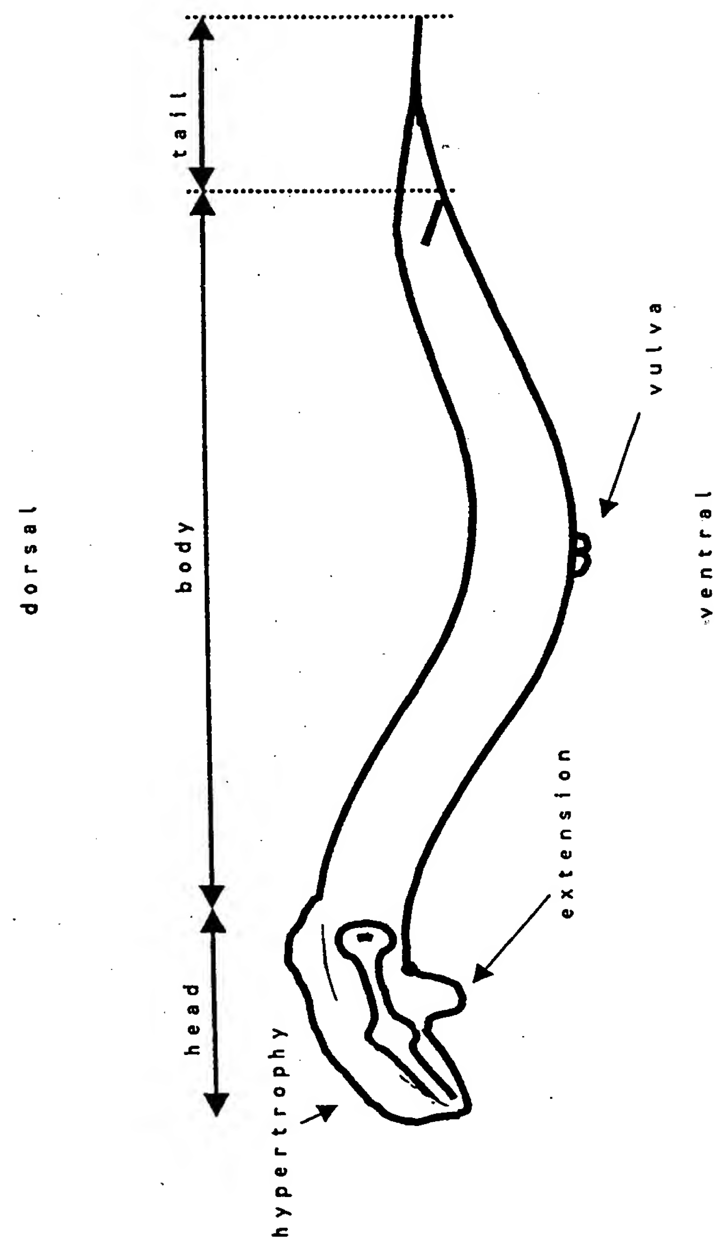

dorsal
tail
body
head
hypertrophy
extension
vulva
ventral

FIG. 2.

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: METHOD FOR CONSTRUCTING LIBRARIES OF PHENOTYPIC PROFILES

(57) Abstract

Methods are provided for use in constructing libraries of phenotypic profiles in a nematode worm such as *C. elegans*. The methods require measurement of identifiable characteristics of the worm and systematic scoring of these characteristics. Also provided are methods of identifying compounds with potential pharmacological activity, for determining the mode of action of a given compound and for assigning genes to particular biochemical pathways.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/09710

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N1/04 C12N1/00 C12N15/01 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 09096 A (CAMBRIDGE NEUROSCIENCE RES ;HORVITZ HOWARD ROBERT (US)) 23 August 1990 (1990-08-23) Cited against inventions 1 and 2 in their entirety and inventions 3 and 4 insofar as "environmental changes" can also include those changes due to (e.g. toxic) compounds. page 7, line 18 -page 8, line 23 page 15, line 14 - line 30 ---	1-112, 114,115

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2000	25.07.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sprinks, M

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KATSURA ET AL.: "Isolation, characterization and epistasis of fluoride-resistant mutants of Caenorhabditis elegans" GENETICS, vol. 136, 1994, pages 145-154, XP000886900 Cited against invention 1 abstract; tables 1-4 page 145, column 1 -page 146, column 1 ---	1-25,71, 72, 75-89, 96-112, 114,115
X	VAN SWINDEREN ET AL.: "Quantitative trait loci controlling halothane sensitivity in Caenorhabditis elegans" PROC. NATL. ACAD. SCI. USA, vol. 94, 1997, pages 8232-8237, XP002137784 Cited against invention 2 in its entirety and invention 3 insofar as "environmental changes" can also include those changes due to (e.g. toxic) compounds. abstract page 8232, column 1 -page 8233, column 1 ---	1-25,71, 72, 75-89, 96-112, 114,115
A	AHRINGER ET AL.: "Turn to the worm!" CURRENT OPINION IN GENETICS AND DEVELOPMENT, vol. 7, 1997, pages 410-415, XP000886904 cited in the application Cited for all inventions the whole document ---	1-112, 114,115
X	WO 96 38555 A (BOGAERT THIERRY ;STRINGHAM EVE (CA); VANDEKERCKHOVE JOEL (BE)) 5 December 1996 (1996-12-05) Cited against inventions 2 and 3 page 35, line 22 -page 36, line 28; claim 43 ---	26-68, 71-77, 79-81, 90-114
A	SAMOILOFF, M.R. ET AL: "The use of nematodes in marine ecotoxicology. ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. VOL. 1." MAR. TOX., (1984) PP. 407-426. MEETING INFO.: INTERNATIONAL SYMPOSIUM ON ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. GHENT (BELGIUM). 12-14 SEP 1983. ISSN: 90-9000814-4;,90-9000812-8., XP000886947 Dep. Zool., Univ. Manitoba, Winnipeg, Man. R3T 2N2, Canada Cited for inventions 3 and 4 page 413, paragraph 2 ---	55-68, 71,72, 75, 79-81, 93-112, 114,115

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3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/09710

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BOGAERT, T; ET AL: "Determination of the toxicity of four heavy metal compounds and three carcinogens using two marine nematode species, Monhystera microphthalma and Diplolaimelloides bruciei. ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. VOL. 2." MAR. TOX., (1984) PP. 21-30. MEETING INFO.: INTERNATIONAL SYMPOSIUM ON ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. GHENT (BELGIUM). 12-14 SEP 1983. ISSN: 90-9000814-4;,90-9000813-6., XP000886948 Lab. Mol. Biol., Med. Res. Counc. Cent., University Med. Sch., Hills Rd., Cambridge CB2 2QH, UK Cited for inventions 3 and 4 the whole document -----	55-68, 71,72, 75, 79-81, 93-112, 114,115

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

BNSDOCID: <WO___0034438A3_I_>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/09710

Box I Obs rvations where c rtain claims were found unsearchable (C ntinuati n f item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 113
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

It is not possible to carry out a meaningful search into the state of the art on the basis of claim 113 because its subject-matter ("agonists" and "antagonists") is structurally undefined and could not in any event have been functionally tested in the prior art.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-25,78,82-89 completely; 71,72,75-77,79-81, 96-115 partially

   Method for determining the mode of action of a compound or gene, comprising comparing the phenotypic response of a nematode treated with said compound or with a defect in said gene with a library of multiple phenotypic traits of nematodes with genetic defects and subject-matter relating thereto.

2. Claims: 26-54,73,74,90-92 completely; 71,72,75-77,79-81, 96-115 partially

   Method for determining the mode of action of a compound, comprising comparing the phenotypic response of a nematode treated with the compound with a library of multiple phenotypic responses of nematodes treated with other compounds and subject-matter relating thereto.

3. Claims: 55-68,93-95 completely; 71,72,75,79-81, 96-115 partially

   Method for determining the mode of action of a compound, comprising comparing the phenotypic response of a nematode treated with the compound with a library of multiple phenotypic responses of nematodes subjected to environmental changes and subject-matter relating thereto.

4. Claims: 69,70 completely; 71,72,75,79-81,96-113 partially

   Method for determining the mode of action of a compound or gene, comprising the methods of inventions 1-3 referred to above and subject-matter relating thereto.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 113

It is not possible to carry out a meaningful search into the state of the art on the basis of claim 113 because its subject-matter ("agonists" and "antagonists") is structurally undefined and could not in any event have been functionally tested in the prior art.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/09710

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9009096 A	23-08-1990	AU 5106790 A	05-09-1990
WO 9638555 A	05-12-1996	AU 6123496 A EP 0832222 A	18-12-1996 01-04-1998